



Effect of Gen (HLA-DR4) on Reumatoide Arthrites In Wasit Province \ Iraq

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Abstract

Rheumatoid arthritis (RA) is a multifaceted inflammatory disease with a significant hereditary component to its pathophysiology. Most of the population is vulnerable to this illness due to genetic factors. Predisposition to RA has been demonstrated to be influenced by genetic variables. Human Leukocyte Antigen –DR isotype 4 system is a gene complex in humans that encodes cell-surface proteins responsible for the regulation of the immune system. HLA-DR is a class of cell-surface receptors encoded for by the HLA system. 90 subjects of both genders were collected, including 60 patients and 30 from the control group. The results showed that the mean levels was lower in RA patients in compared to healthy control and the difference was highly significant ($P < 0.001$). The studied genome contribute as indicators of the development of health risks to the body and Reumatoide Arthrites health and patients.

Keywords: Reumatoide Arthrites; Human Leukocyte Antigen –DR isotype 4 (HLA-DR4).

Introduction

Rheumatoid Arthritis (RA) is a multifaceted inflammatory disease with a significant hereditary component to its pathophysiology. Most of the population is vulnerable to this illness due to genetic factors. Predisposition to RA has been demonstrated to be influenced by genetic variables. Variation (or polymorphism) of the genes encoding numerous proteins known to be highly implicated in driving the inflammatory process in RA has been discovered in studies (1). The human leukocyte antigen (HLA) system is a gene complex in humans that encodes cell-surface proteins responsible for the regulation of the immune system. HLA-DR is a class of cell-surface receptors encoded for by the HLA system. Estimating the role of some genome parameters in RA patients such Human leukocyte Antigen (HLA-DR4),

Materials and methods

2.1 Study Subjects

This study was conducted at the Department of Biology - College of Science / Wasit University in cooperation with Al-Zahra Teaching Hospital of the Wasit Health Department, during the period from November, 2024 to March, 2025 - . The study included 90 samples, whose ages ranged from 18 to 70 years, 60 patients of both gender, who were clinically diagnosed with RA (based on age, gender and Duration of disease) by a specialized physician and 30 healthy control. and verbal consent was obtained from the participants and they all agreed to contribute to the study. Samples (Molecular) were excluded.

2.2: Methods

2 ml of venous blood was drawn for both healthy and sick patients, , 2ml of blood was placed In gel tubes to obtain blood serum for measurement of indicators of Reumatoide Arthrites.

Blood samples were examined using the America-made Fine device, according to the manufacturer's instructions (Fine).The values of blood parameters were calculated for both patients and healthy subjects,by and the readings is recorded. Where the levels of Human leukocyte Antigen (HLA-DR4).) was calculated.

2.3 Statistical analysis

The statistical analysis of the current study was based on (SPSS) version 26, which was based on the value of (Mean $\pm$ SD) to detect the effect of variation factors in the study parameters, using (ANOVA) method was used to find the least significant difference. (LSD) is used to compare the two groups (patients and control) within the tests studied under the probability level ($P \geq 0.05$),

Results and discussion

3.1. Result of Real time PCR Quantification of DR-4 gene Expression

The mean Ct value of DR-4 cDNA amplification was (27.35) in the RA patients. While Ct values in control were mean (25.62), the mean Ct values in control group were lower than those of RA patients. “This is important in reflecting the original DR-4 present in the samples. It is evident from the results that patients group is associated with the highest copy number of DR-4 reflecting its lower expression.

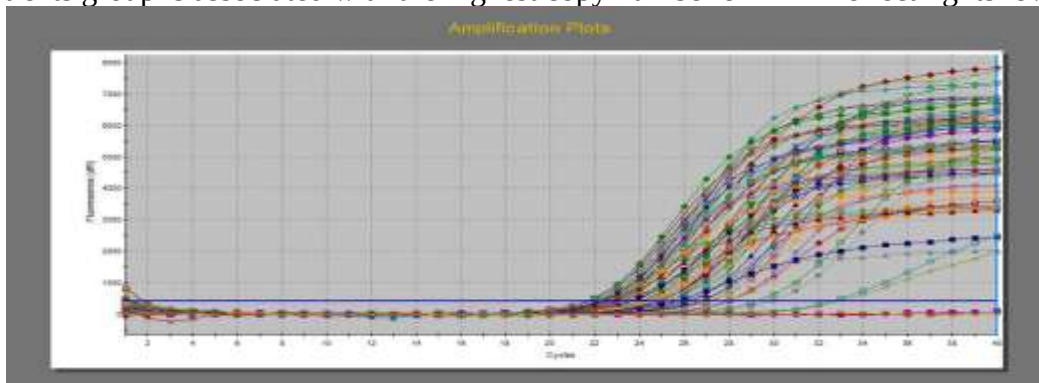


Figure (1-1) DR-4 gene amplification plots by qPCR. Samples included all study groups. The photograph was taken directly from Agilent qPCR machine.

In this study, a quantitative analysis of RT-PCR analyzed *Expression of comparison DR-4* and its expression between, apparently control group, the group RA group. The change in gene expression was calculated using a relative quantitative measurement (2). This is based on the normalization of the Ct values for calculating ΔCt and represents the difference between the average Ct values of the DR-4 cDNA amplification replica for each case and case of *GAPDH*.

In calculation of the relative expression of *DR-4 gene* in all study groups the Fold number results was applied. A calibrator was used and it was one of the samples of the controls with high expression of *DR-*

4. When calculating, the gene expression was significantly lower in RA patients group than control group. Fold number in RA group was 0.472, as shown in table (1-1).

Table (1-1): Comparison of (Ct, $2^{-\Delta Ct}$ and Folding) between patients and healthy controls

Groups	Means Ct of DR-4	Means Ct of GAPDH	^4Ct (Means Ct of DR-4)	Fold of gene expression
RA patients	27.35	23.19	4.15	0.472
Control	25.62	23.62	1.99	1.00

The comparison of *DR-4* gene expression between RA patients and healthy control subjects has been carried out and the results were demonstrated in table (1-2). Mean of *DR-4* gene expression were 0.472 ± 0.19 , and 1.00 ± 0.28 in RA patients and healthy control respectively; the mean levels was lower in RA patients in compared to healthy control and the difference was highly significant ($P < 0.001$).

Table (1-2): Comparison of mean of *DR-4* gene expression between patients and healthy controls

Groups	Mean	SD	SE	p-value
RA patients	0.472	0.19	0.06	0.001**
Control	1.00	0.28	0.11	

SD: standard deviation; SE: standard error; †: one way ANOVA; **: significant at $P < 0.05$

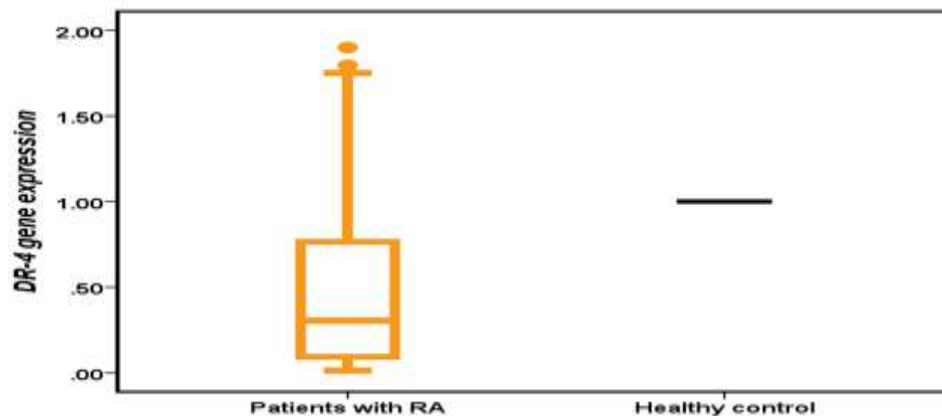


Figure (1-2): The means *DR-4* gene expression in patients and control groups

The observed decrease in *DR-4* gene expression among patients with rheumatoid arthritis (RA) may suggest a modified immune response linked to the condition. As a component of the human leukocyte antigen (HLA) system, *DR-4* is essential for the immune system's capacity to identify and react to foreign antigens. The results indicating reduced *DR-4* expression in RA patients align with earlier research that proposes immune dysregulation, especially concerning antigen-presenting cells and T-cell activation, plays a significant role in the development of RA (3).

In a comparable investigation conducted by (4), a reduced expression of *DR-4* was also observed in individuals suffering from autoimmune disorders such as rheumatoid arthritis, thereby reinforcing the notion that *DR-4* may be integral to the pathogenesis of RA. The implications of *DR-4* downregulation suggest its potential utility as a biomarker for RA or as a target for therapeutic intervention in the management of the disease. Subsequent research could explore the molecular mechanisms that underlie this diminished expression and assess whether the restoration of *DR-4* levels could yield therapeutic benefits for patients with RA. Furthermore, variations in HLA-DR expression, including that of *DR-4*, have been shown to correlate with the severity of autoimmune conditions, including RA. It was noted that the decreased expression of HLA-DR in RA patients might be associated with dysregulation of the immune system and the loss of tolerance (4,5).

Research conducted in studies of (6) examined gene expression profiles in patients with rheumatoid arthritis (RA) and identified that variations in the expression of the HLA-DR gene may serve as biomarkers for the severity and progression of the disease. The results indicate that diminished expression of DR-4 could hinder antigen presentation, a critical process in the formation of autoimmune responses. (6).

3.2. Diagnostic accuracy of *DR-4 gene expression*

Receiver operating characteristic (ROC) analysis was performed to reveal the diagnostic accuracy of using *DR-4 gene* to distinguish RA patients from healthy control subjects. An optimal *DR-4 gene* cut-off value less than of 0.988 resulted in an AUC value of 0.867 (95% confidence interval [CI], 0.781- 0.953, $P = 0.001$), sensitivity of 86.7%, specificity of 100.0%, PPV of 100.0%, and NPV of 78.9%. The present results indicates *DR-4 gene* is considered as good diagnostic marker to distinguish RA patients from healthy control.

Table (1-3): Roc curve of *DR-4 gene expression*

Characteristic	RA patients / control
Cutoff value	< 0.988
P value	0.001
Sensitivity %	86.7 %
Specificity %	100.0 %
PPV %	100.0 %
NPV %	78.9 %
AUC (95% CI)	0.867 (0.781- 0.953)

CI: Confidence interval, AUC: Area under curve.

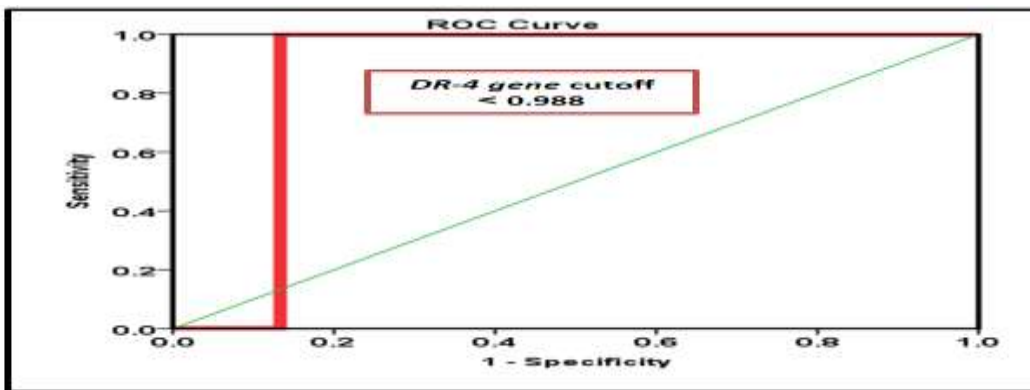


Figure (1-3): Receiver operating characteristic curve for *DR-4 gene* to distinguish RA patients from healthy control subjects.

Gene expression profiling conducted on synovial tissue fibroblast cells reveals that numerous genes, notably those within the HLA family, exhibit expression alterations associated with rheumatoid arthritis (RA) disease activity. In particular, the expression of HLA-DQA2 is found to correlate with functional disability scores, while Clec12A shows a relationship with rheumatoid factor levels. This suggests that the expression of HLA-related genes, such as DR-4, may yield diagnostic insights by mirroring disease mechanisms and clinical parameters (7). These gene signatures provide a level of specificity that exceeds

that of traditional serological markers, which, although beneficial, may not possess adequate sensitivity or prognostic capability when considered in isolation (8; 9).

Comprehensive meta-analyses of large gene expression datasets from patients with rheumatoid arthritis (RA) demonstrate a consistent differential expression of immune-related genes, notably including HLA class II molecules akin to DR-4, which are integral to antigen presentation essential for the pathogenesis of RA. The upregulation of these genes underscores their potential utility not only in diagnosis but also in elucidating disease progression and possibly in therapeutic targeting (10). This finding serves to enhance serological diagnostics, such as rheumatoid factor isotyping and testing for anti-citrullinated protein antibodies (ACPA), which, while significant, exhibit variability in sensitivity and specificity across different assays (8; 9).

The role of DR-4 gene expression in rheumatoid arthritis (RA) underscores its diagnostic accuracy, as it contributes to disease-specific immune gene signatures that correlate with disease activity and offer molecular specificity that exceeds traditional antibody tests. Incorporating this gene expression into diagnostic practices has the potential to improve early detection and tailor treatment strategies to individual patients; however, additional harmonization and validation within clinical environments are essential to establish reliable diagnostic standards. (7;10; 11).

3.3. Frequency distribution of DR-4 gene according to duration of disease

The comparison of *DR-4 gene* according to duration of disease has been carried out and the results were demonstrated in table (1-4). In RA patients, the present results show there was non-significant difference between male patients and female patients according to *DR-4 gene expression*, ($p>0.05$).

Table (1-4): Frequency distribution of DR-4 gene according to gender

Characteristics		DR-4 gene expression
Duration of disease		
< 5 years	Mean $\pm$ SD	0.511 $\pm$ 0.13
$\geq$ 5 years	Mean $\pm$ SD	0.404 $\pm$ 0.11
p-value		0.421 †

SE: standard Error; †: Independent T test; **: significant at $P < 0.05$

The absence of a notable difference may suggest that the expression of the DR-4 gene remains relatively consistent over time concerning the duration of the disease. This could indicate that the factors influencing the gene's expression are more likely to be related to elements other than the length of the disease's presence, such as genetic predisposition or various biological factors. Research regarding gene expression in the context of disease progression frequently emphasizes how certain genes can be affected by both the duration and severity of the disease; however, in this instance, the DR-4 gene does not exhibit such a trend. This observation implies either a potentially limited role of the gene in disease progression or a stable expression profile throughout different stages of the disease (12).

When examining gender, it is important to note that while the available data does not explicitly delineate DR-4 expression by sex, existing literature generally indicates that gene expression can vary between males and females due to biological and hormonal factors. Documented differences in gene expression related to gender have been observed across various diseases and tissues, which can influence susceptibility to diseases, their progression, and subsequent outcomes. Such patterns of differential expression may be regulated by sex chromosomes and hormones, as evidenced by studies demonstrating that males and females display unique gene expression profiles in the context of disease. (13; 14).

Conclusion

The present study revealed that the levels of reduction in DR-4 gene expression in RA patients may be indicative of an altered immune response associated with the disease. The present results indicates *DR-4 gene* is considered as good diagnostic marker to distinguish RA patients from healthy control.

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